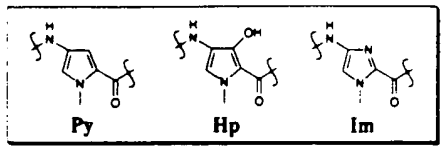
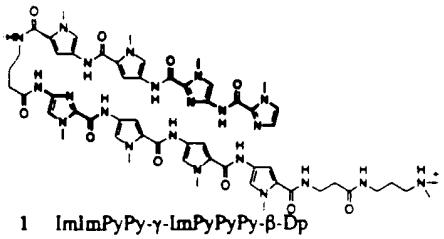
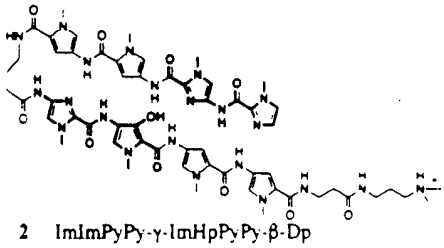
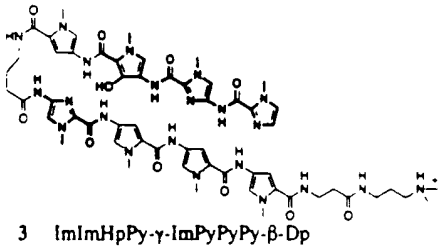




1 ImImPyPy-γ-ImPyPyPy-β-Dp



2 ImImPyPy-γ-ImHpPyPy-β-Dp



3 ImImHpPy-γ-ImPyPyPy-β-Dp

FIG. 1

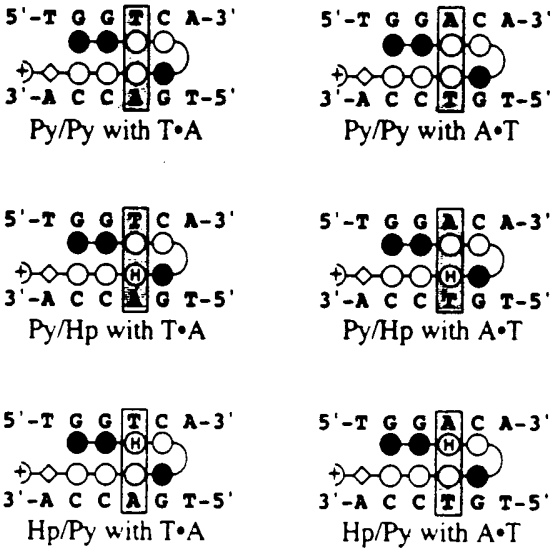

5'-T G G T C A-3'
3'-A C C A G T-5'
Py/Py with T•A
5'-T G G A C A-3'
3'-A C C T G T-5'
Py/Py with A•T
5'-T G G T C A-3'
3'-A C C A G T-5'
Py/Hp with T•A
5'-T G G A C A-3'
3'-A C C T G T-5'
Py/Hp with A•T
5'-T G G T C A-3'
3'-A C C A G T-5'
Hp/Py with T•A
5'-T G G A C A-3'
3'-A C C T G T-5'
Hp/Py with A•T

FIG. 2

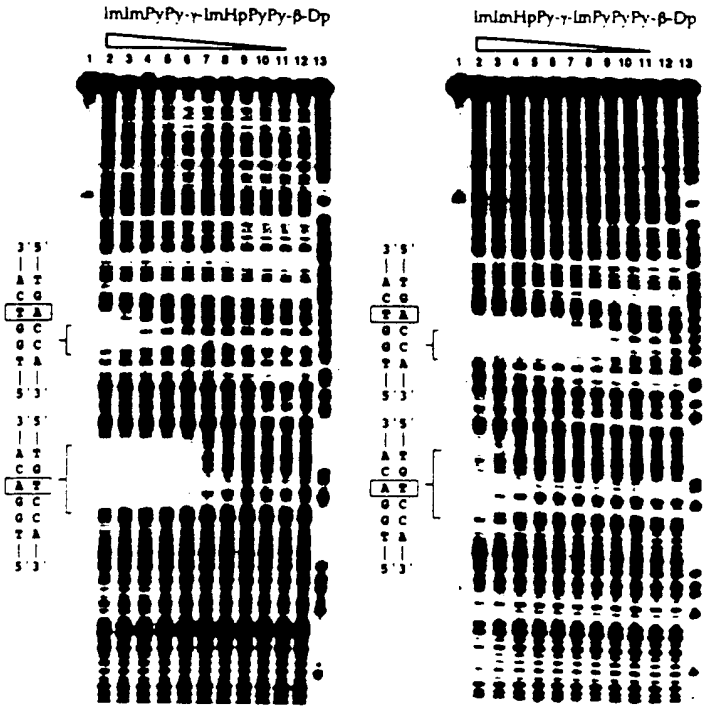
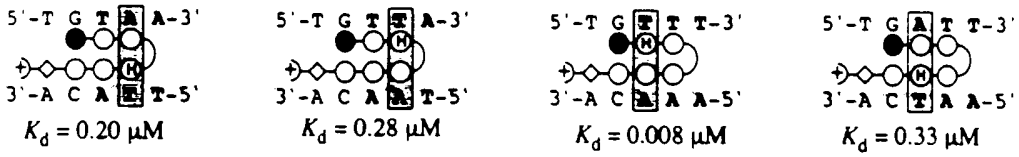
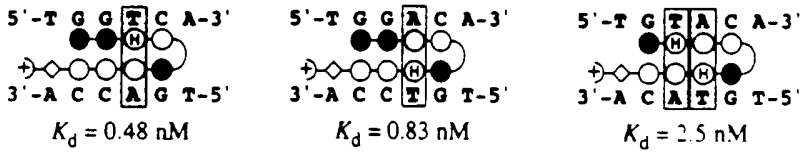

ImImPyPy-γ-ImHpPyPy-β-Dp
ImImHpPy-γ-ImPyPyPy-β-Dp
1 2 3 4 5 6 7 8 9 10 11 12 13

FIG. 3

6-Ring Hairpin Hp-Py-Im-Polyamides



8-Ring Hairpin Hp-Py-Im-Polyamides



10-Ring Hairpin Hp-Py-Im-Polyamides

FIG. 4

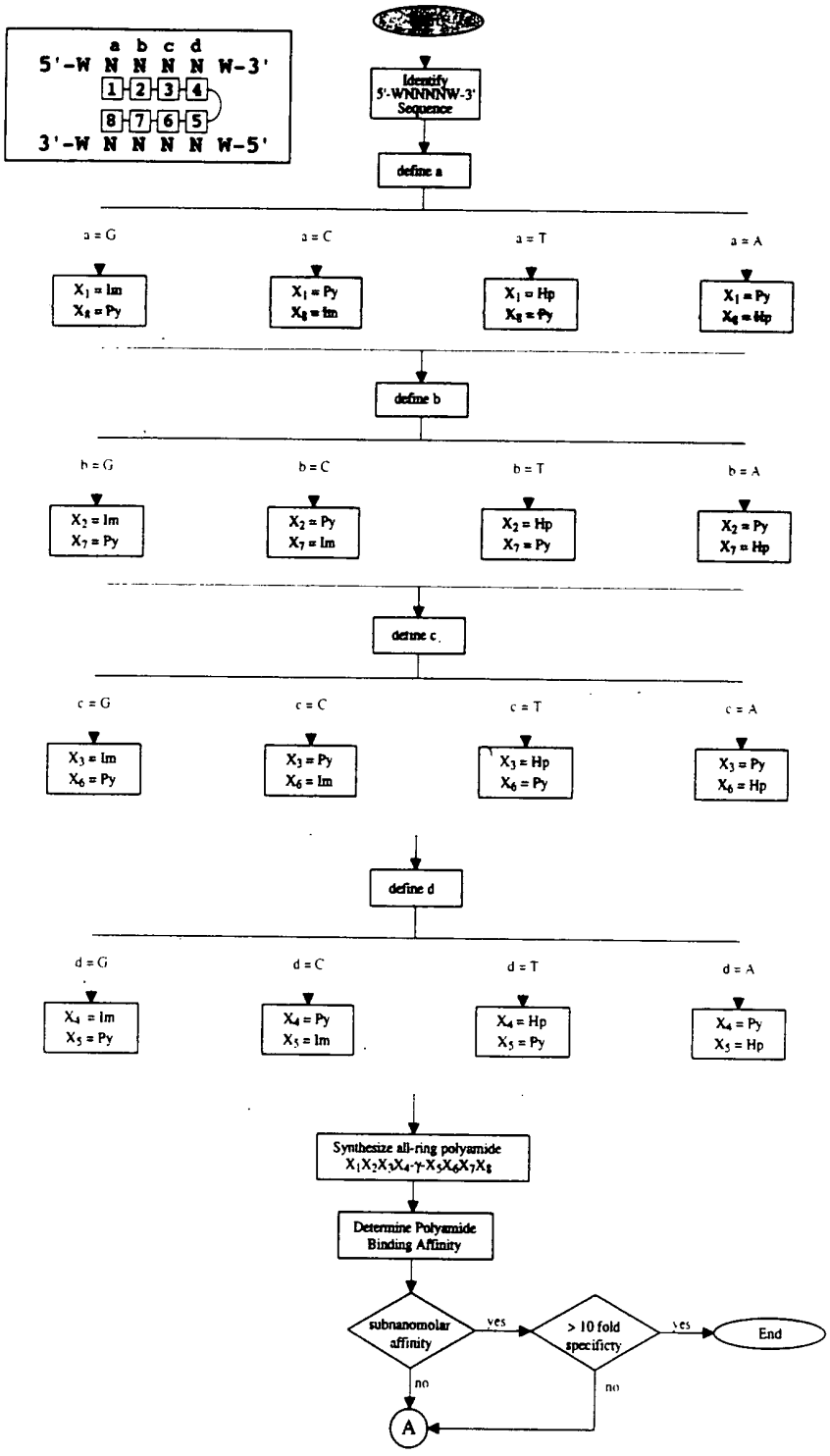

a b c d
5'-W N N N N W-3'
1 2 3 4
8 7 6 5
3'-W N N N N W-5'
Identify 5'-WNNNNW-3' Sequence
define a
a = G
a = C
a = T
a = A
X1 = Im X8 = Py
X1 = Py X8 = Im
X1 = Hp X8 = Py
X1 = Py X8 = Hp
define b
b = G
b = C
b = T
b = A
X2 = Im X7 = Py
X2 = Py X7 = Im
X2 = Hp X7 = Py
X2 = Py X7 = Hp
define c
c = G
c = C
c = T
c = A
X3 = Im X6 = Py
X3 = Py X6 = Im
X3 = Hp X6 = Py
X3 = Py X6 = Hp
define d
d = G
d = C
d = T
d = A
X4 = Im X5 = Py
X4 = Py X5 = Im
X4 = Hp X5 = Py
X4 = Py X5 = Hp
Synthesize all-ring polyamide X1X2X3X4-γ-X5X6X7X8
Determine Polyamide Binding Affinity
subnanomolar affinity
yes
> 10 fold specificty
yes
End
no
no
A

FIG. 5

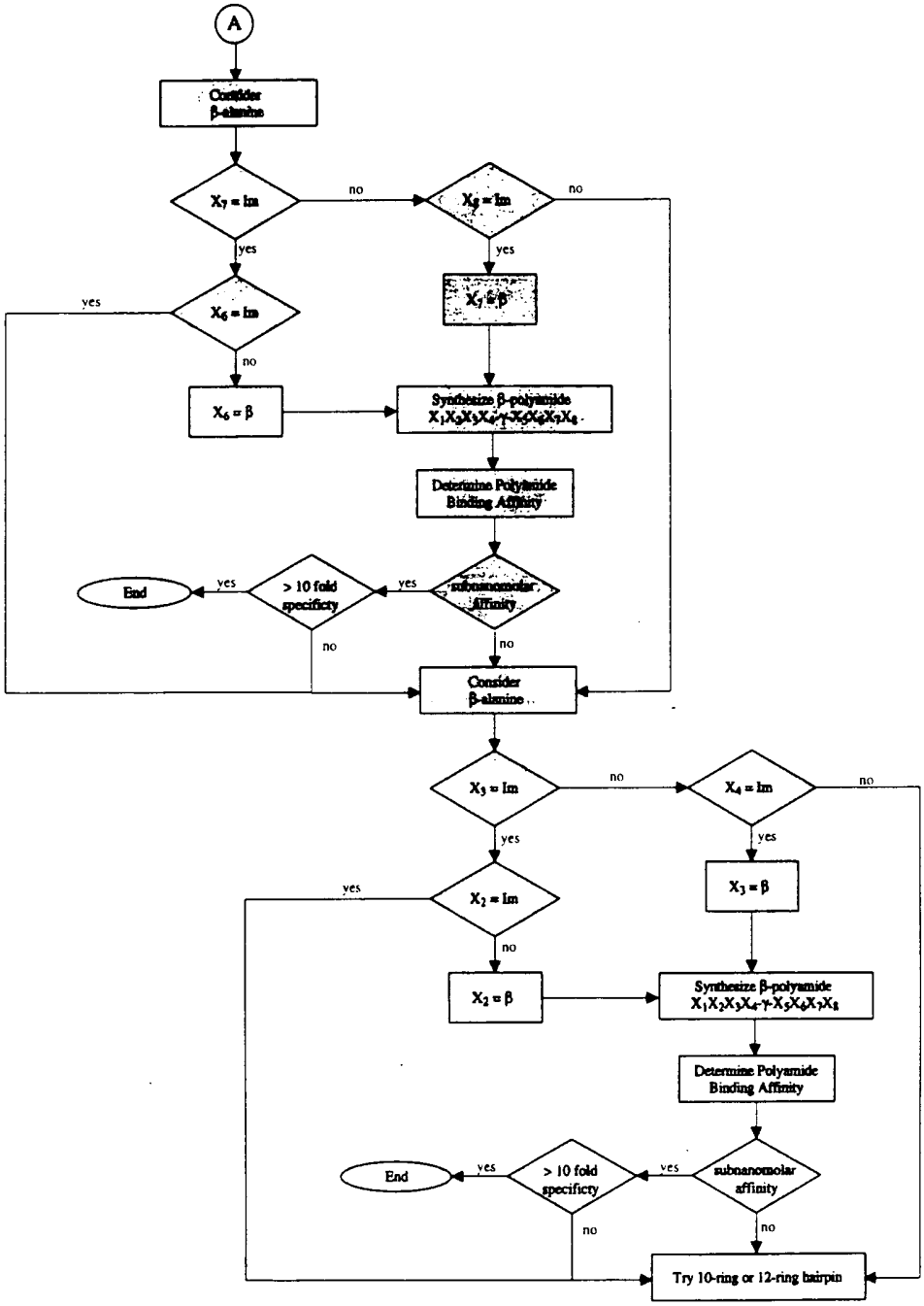

A
Consider β-alanine
X7 = Im
no
X8 = Im
no
yes
yes
X6 = Im
yes
X7 = β
no
X6 = β
Synthesize β-polyamide
X1X2X3X4-γ-X5X6X7X8
Determine Polyamide Binding Affinity
End
yes
> 10 fold specificity
yes
subnanomolar Affinity
no
no
Consider β-alanine
X3 = Im
no
X4 = Im
no
yes
yes
yes
X2 = Im
X3 = β
no
X2 = β
Synthesize β-polyamide
X1X2X3X4-γ-X5X6X7X8
Determine Polyamide Binding Affinity
End
yes
> 10 fold specificty
yes
subnanomolar affinity
no
no
Try 10-ring or 12-ring hairpin

FIG. 6

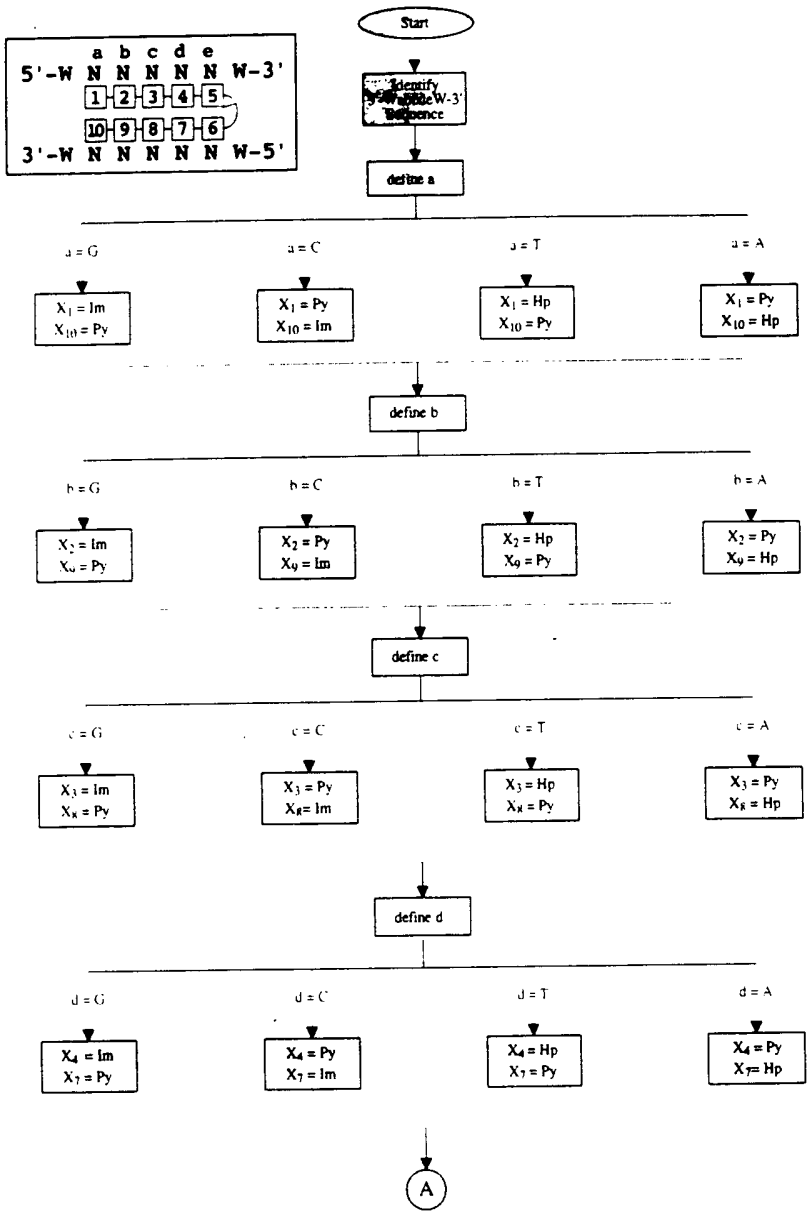

a b c d e
5'-W N N N N N W-3'
1 2 3 4 5
10 9 8 7 6
3'-W N N N N N W-5'
Start
Identify
W-3'
Reference
define a
a = G
a = C
a = T
a = A
X1 = Im
X10 = Py
X1 = Py
X10 = Im
X1 = Hp
X10 = Py
X1 = Py
X10 = Hp
define b
b = G
b = C
b = T
b = A
X2 = Im
X9 = Py
X2 = Py
X9 = Im
X2 = Hp
X9 = Py
X2 = Py
X9 = Hp
define c
c = G
c = C
c = T
c = A
X3 = Im
X8 = Py
X3 = Py
X8 = Im
X3 = Hp
X8 = Py
X3 = Py
X8 = Hp
define d
d = G
d = C
d = T
d = A
X4 = Im
X7 = Py
X4 = Py
X7 = Im
X4 = Hp
X7 = Py
X4 = Py
X7 = Hp
A

FIG. 7a

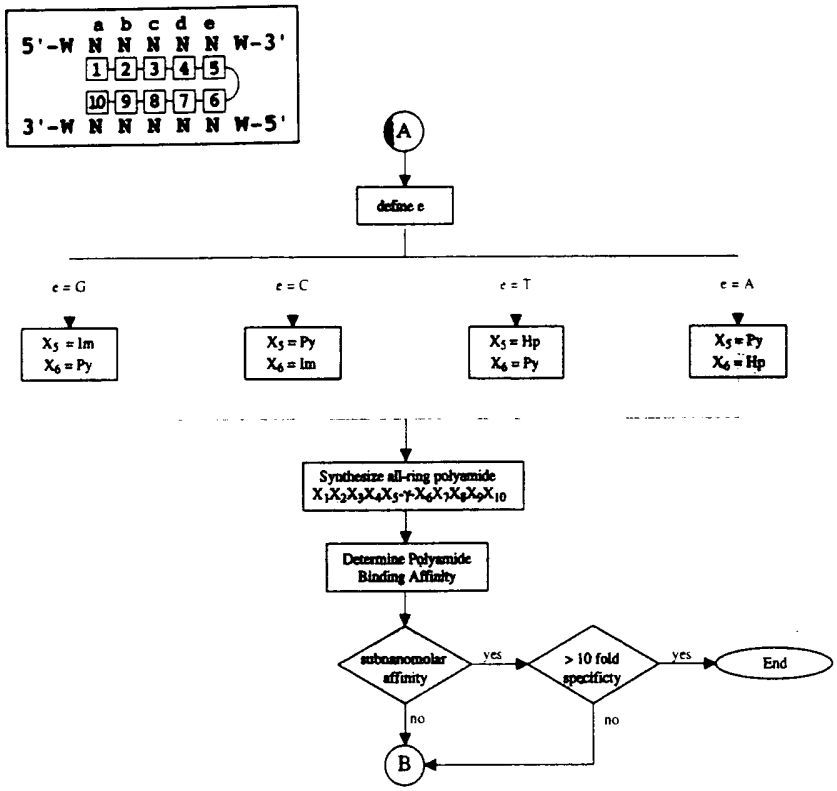

a b c d e
5'-W N N N N N W-3'
1 2 3 4 5
10 9 8 7 6
3'-W N N N N N W-5'
A
define e
e = G
e = C
e = T
e = A
X5 = Im
X6 = Py
X5 = Py
X6 = Im
X5 = Hp
X6 = Py
X5 = Py
X6 = Hp
Synthesize all-ring polyamide
X1X2X3X4X5-γ-X6X7X8X9X10
Determine Polyamide Binding Affinity
subnanomolar affinity
yes
> 10 fold specificty
yes
End
no
no
B

FIG. 7b

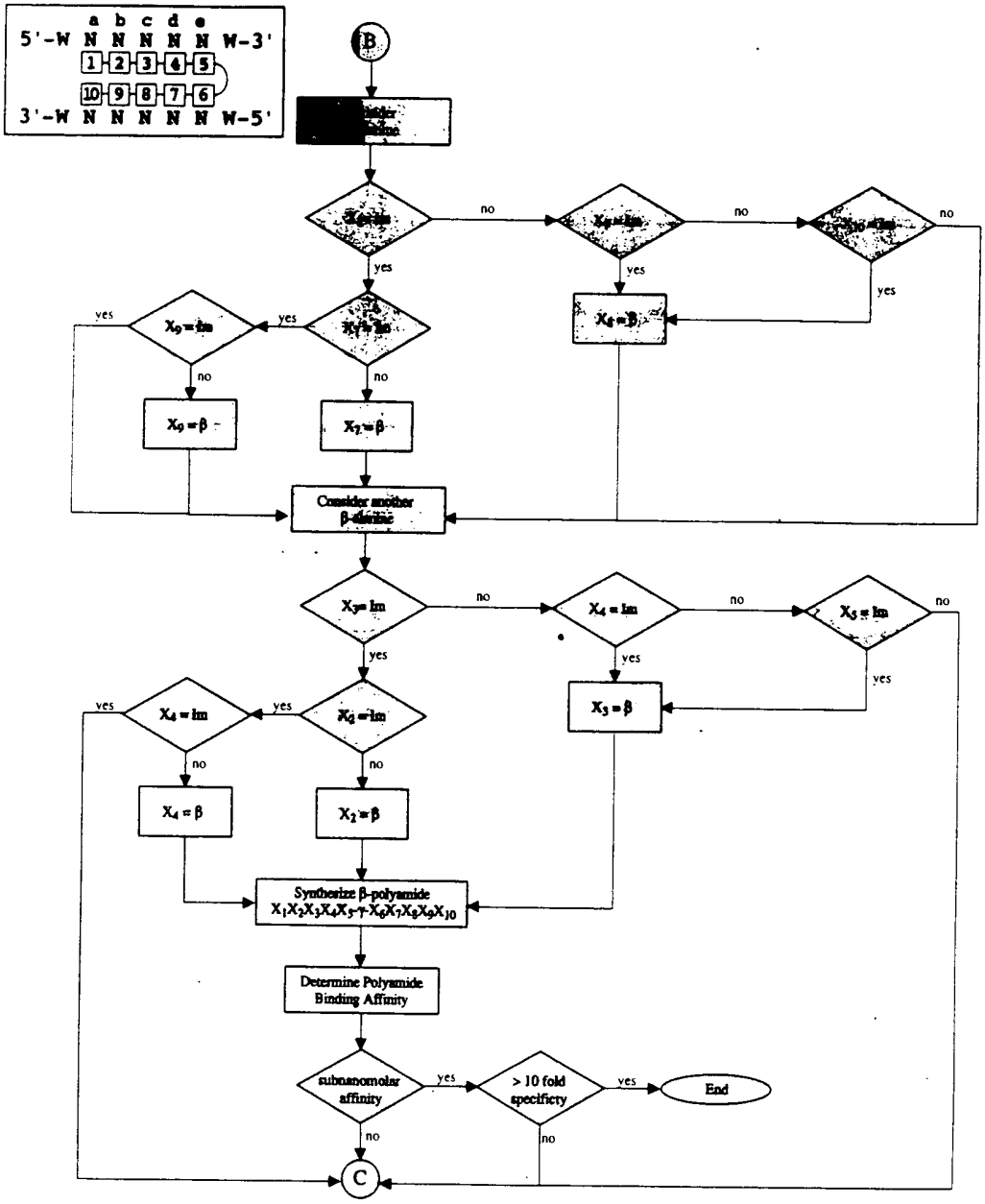

a b c d e
5'-W N N N N N W-3'
1 2 3 4 5
10 9 8 7 6
3'-W N N N N N W-5'
B
no
yes
Consider another β-diastase
Synthesize β-polyamide
X1X2X3X4X5-γ-X6X7X8X9X10
Determine Polyamide Binding Affinity
subnanomolar affinity
> 10 fold specificty
End
C

FIG. 8

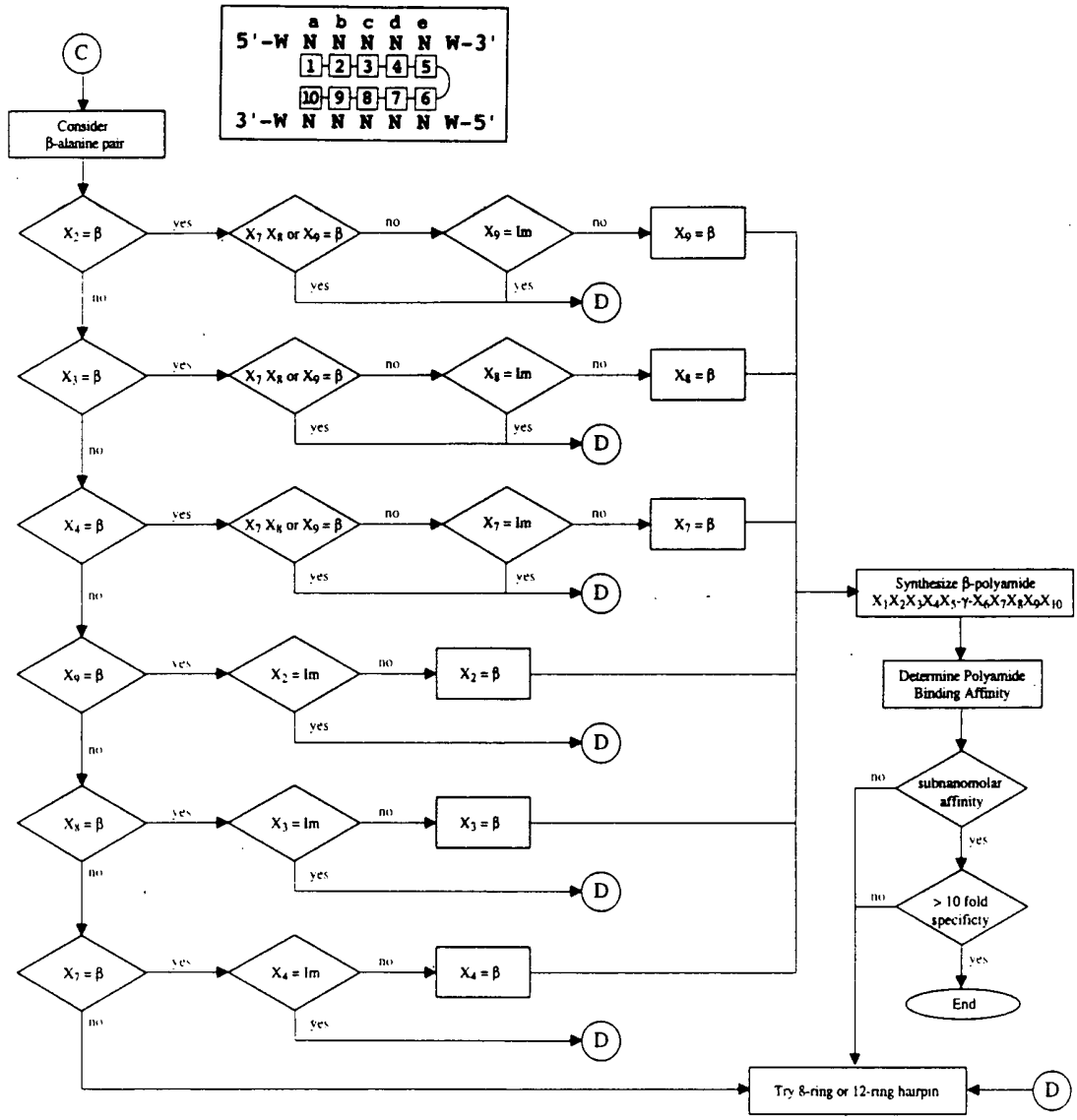

a b c d e
5'-W N N N N N W-3'
1 2 3 4 5
10 9 8 7 6
3'-W N N N N N W-5'
C
Consider β-alanine pair
X2 = β
X7 X8 or X9 = β
X9 = Im
X9 = β
X3 = β
X8 = Im
X8 = β
X4 = β
X7 = Im
X7 = β
X9 = β
X2 = Im
X2 = β
X8 = β
X3 = Im
X3 = β
X7 = β
X4 = Im
X4 = β
yes
no
D
Synthesize β-polyamide
X1X2X3X4X5-γ-X6X7X8X9X10
Determine Polyamide Binding Affinity
subnanomolar affinity
> 10 fold specificty
End
Try 8-ring or 12-ring hairpin

FIG. 9

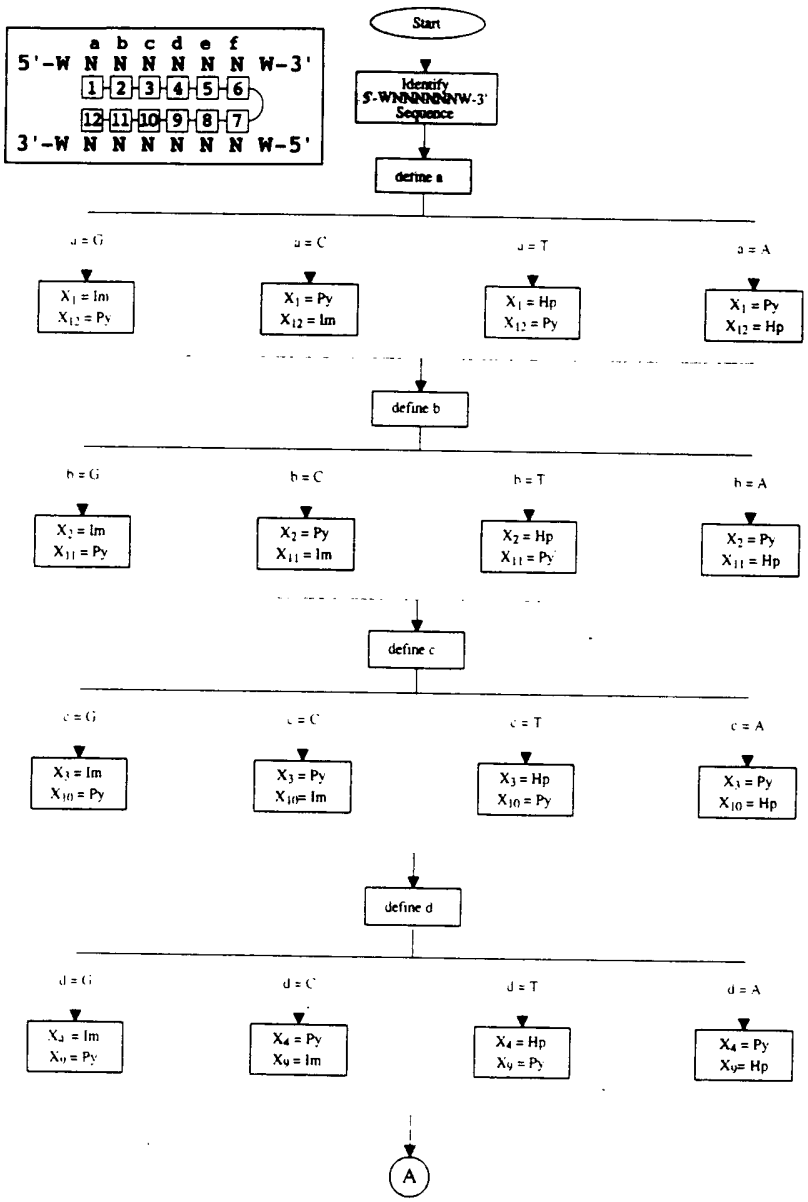

a b c d e f
5'-W N N N N N N W-3'
1 2 3 4 5 6
12 11 10 9 8 7
3'-W N N N N N N W-5'
Start
Identify 5'-WNNNNNNW-3' Sequence
define a
a = G
X1 = Im
X12 = Py
a = C
X1 = Py
X12 = Im
a = T
X1 = Hp
X12 = Py
a = A
X1 = Py
X12 = Hp
define b
b = G
X2 = Im
X11 = Py
b = C
X2 = Py
X11 = Im
b = T
X2 = Hp
X11 = Py
b = A
X2 = Py
X11 = Hp
define c
c = G
X3 = Im
X10 = Py
c = C
X3 = Py
X10= Im
c = T
X3 = Hp
X10 = Py
c = A
X3 = Py
X10 = Hp
define d
d = G
X4 = Im
X9 = Py
d = C
X4 = Py
X9 = Im
d = T
X4 = Hp
X9 = Py
d = A
X4 = Py
X9= Hp
A

FIG 10A

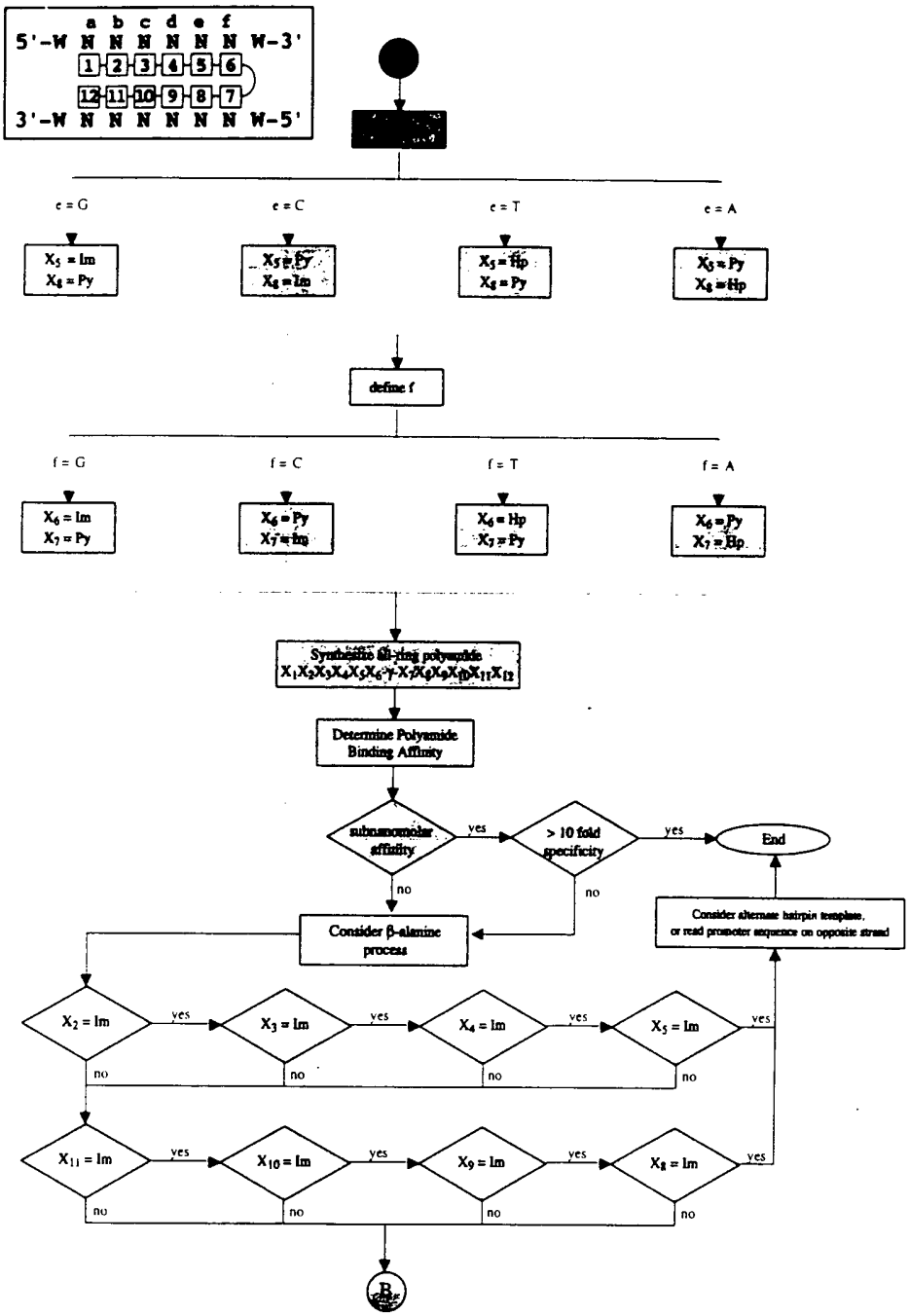

a b c d e f
5'-W N N N N N N W-3'
1 2 3 4 5 6
12 11 10 9 8 7
3'-W N N N N N N W-5'
e = G
e = C
e = T
e = A
X5 = Im
X8 = Py
X5 = Py
X8 = Im
X5 = Hp
X8 = Py
X5 = Py
X8 = Hp
define f
f = G
f = C
f = T
f = A
X6 = Im
X7 = Py
X6 = Py
X7 = Im
X6 = Hp
X7 = Py
X6 = Py
X7 = Hp
Determine Polyamide Binding Affinity
subnanomolar affinity
yes
> 10 fold specificity
yes
End
no
no
Consider β-alanine process
Consider alternate hairpin template, or read promoter sequence on opposite strand
X2 = Im
X3 = Im
X4 = Im
X5 = Im
X11 = Im
X10 = Im
X9 = Im
X8 = Im
yes
no

FIG 10B

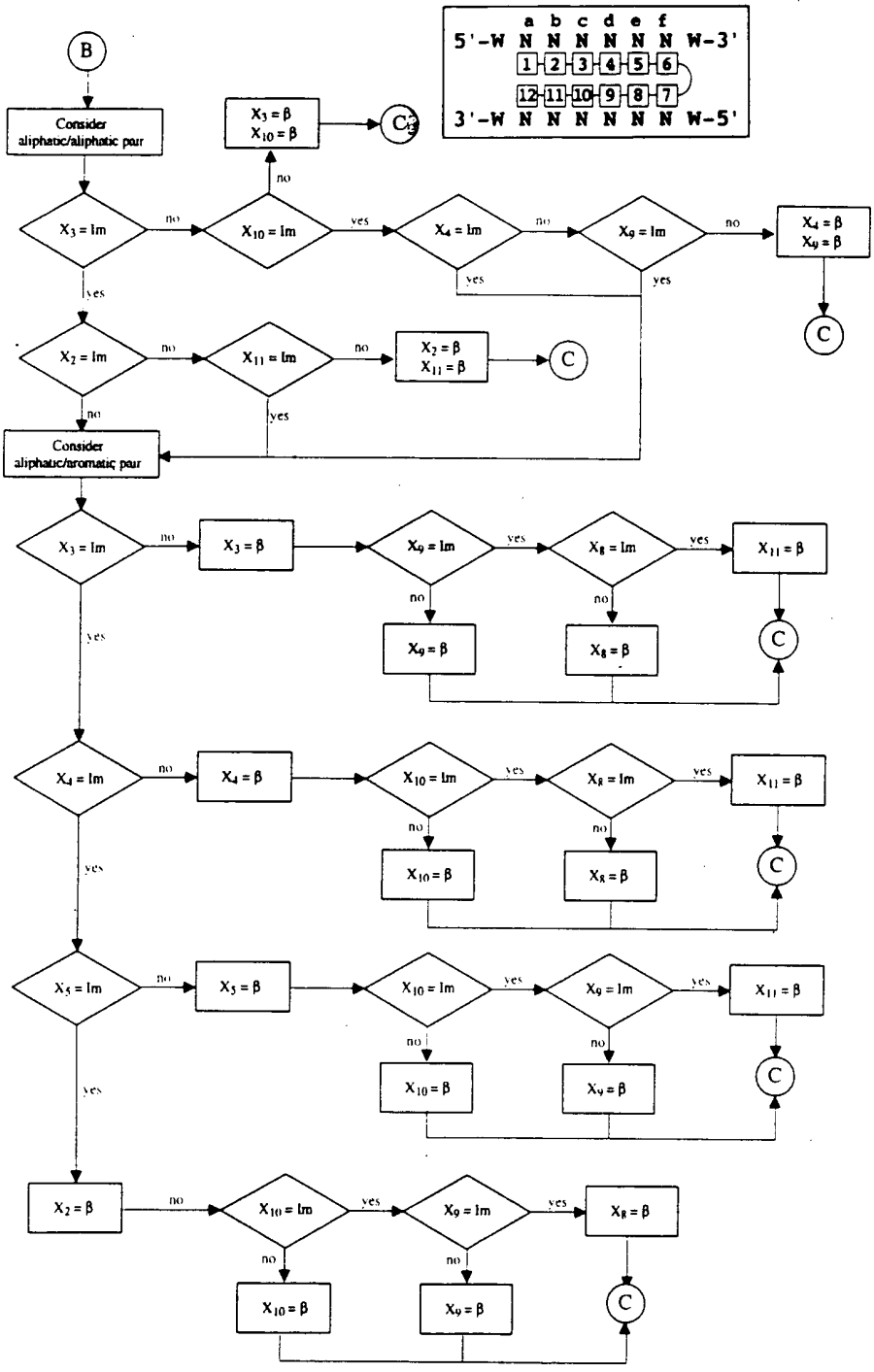

a b c d e f
5'-W N N N N N N W-3'
1 2 3 4 5 6
12 11 10 9 8 7
3'-W N N N N N N W-5'
B
Consider aliphatic/aliphatic pair
X3 = β
X10 = β
C
X3 = Im
X10 = Im
X4 = Im
X9 = Im
X4 = β
X9 = β
X2 = Im
X11 = Im
X2 = β
X11 = β
Consider aliphatic/aromatic pair
X8 = Im
X11 = β
X9 = β
X8 = β
X5 = Im
X5 = β
X10 = β
X2 = β
no
yes

FIG 11A

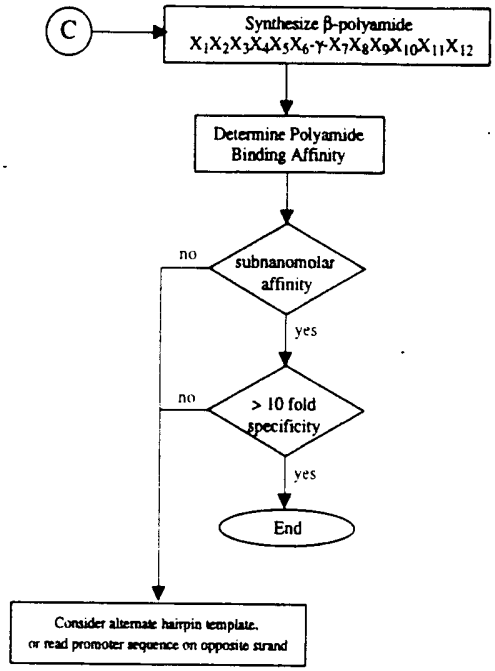

C
Synthesize β-polyamide
$X_1X_2X_3X_4X_5X_6$-γ-$X_7X_8X_9X_{10}X_{11}X_{12}$
Determine Polyamide Binding Affinity
no
subnanomolar affinity
yes
no
> 10 fold specificity
yes
End
Consider alternate hairpin template, or read promoter sequence on opposite strand

FIG 11B